The role of fluoride and casein phosphopeptide/amorphous calcium phosphate in the prevention of erosive/abrasive wear in an in vitro model using hydrochloric acid

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The Role of Fluoride and Casein Phosphopeptide/Amorphous Calcium Phosphate in the Prevention of Erosive/Abrasive Wear in an in vitro Model Using Hydrochloric Acid

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Key Words

Casein phosphopeptide/amorphous calcium phosphate · Fluoride · Erosion · Abrasion

Abstract

Objective: To investigate the effect of various fluoride compounds and casein phosphopeptide/amorphous calcium phosphate (CPP-ACP) on the reduction of erosive/abrasive tooth wear. ***Methods:*** Forty enamel samples were prepared from bovine lower incisors, stratified and allocated to 4 groups (1–4). Samples in group 1 remained untreated and served as negative controls. The test samples were treated for 2 min/day as follows: group 2 amine/sodium fluoride gel (pH 4.8; 12,500 ppm), group 3 sodium fluoride gel (pH 7.1; 12,500 ppm) and group 4 CPP-ACP-containing mousse. De- and remineralization cycling was performed for 20 days with 6 erosive attacks for 20 s with HCl (pH 3.0) per day. Samples were stored in artificial saliva between cycles and overnight. Toothbrushing (15 s; 60 strokes/min; load 2.5 N) with a toothpaste slurry was performed each day before the first and 1 h after the last erosive exposure. Tooth wear was measured by comparing baseline surface profiles with the corresponding posttreatment profiles. ***Results:*** Tooth wear was significantly reduced in groups 2 and 3 compared with group 1, while the enamel loss of group 4 was not significantly lower compared to the negative control group 1. Between the fluoride groups 2 and 3, no significant difference in tooth wear was recorded. ***Conclusion:*** Erosive/abrasive tooth wear under the conditions used could be reduced significantly by the daily application of fluoride gels, irrespective of the fluoride compound, while the application of CPP-ACP-containing mousse was less effective.

Introduction

In developed countries, dental hard tissue loss due to caries has declined over the last decades [Petersson and Bratthall, 1996]. Due to this decrease, other reasons for dental hard tissue loss have entered the focus of dental research, namely erosion [Jaeggi and Lussi, 2006]. It is not absolutely clear if the prevalence of dental erosion is rising [Nunn et al., 2003] or if increased awareness about erosion has led to a more precise examination and diagnosis [Lussi et al., 1991; Downer, 1995]. Irrespective of this discussion, erosive tooth wear has become a focus of dental researchers and practitioners [Wiegand and Attin, 2003; Zero and Lussi, 2005]. Dental erosion is defined as tooth wear due to chemical dissolution of dental hard tissues by acids or chelators in the absence of micro-organisms [Zipkin and McClure, 1949]. In addition to the tooth wear caused by chemical dissolution of dental hard tis-

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sues, a softening of the dental hard tissues can be observed, leading to a higher susceptibility due to toothbrush abrasion [Attin et al., 2000a, b]. The erosion-causing acids can be distinguished by their origin into extrinsic or intrinsic factors [Zero, 1996; Zero and Lussi, 2005]. Extrinsic origins of acids are mostly acidic food and beverages [Lussi et al., 2004; Bartlett, 2005] or, less frequently, acidic fumes in chemical or galvanic factories [Tuominen and Tuominen, 1992; Arowojolu, 2001]. The intrinsic origin for acid is gastric juice, with its high concentration of HCl [Hunt, 1951]. During regurgitation of stomach content, highly acidic gastric juice comes into contact with dental hard tissues. Regurgitation of stomach content is observed in patients suffering from anorexia nervosa, bulimia nervosa and gastrointestinal diseases such as gastro-oesophageal reflux disease [Jarvinen et al., 1988; Bartlett and Coward, 2001; Holbrook et al., 2009]. The best approach to prevent erosive tooth wear is primary prevention: the treatment and elimination of causative factors [Lussi and Hellwig, 2006]. In patients suffering from dental hard tissue loss due to gastric acid, the strategy to eliminate the causes of the disease is often challenging and not completely successful. Thus, along with cause-related treatment, these patients require supplemental treatment to minimize dental hard tissue loss.

In order to prevent tooth wear due to erosion, different approaches such as the improvement of remineralization of softened enamel and an increase in acid resistance of enamel by the topical application of fluorides have been discussed [Graubart et al., 1972; Amaechi and Higham, 2005]. For the prevention of caries, it is evident that fluoride promotes the remineralization and inhibits the demineralization of dental hard tissues [Petersson and Bratthall, 1996; Aleksejuniene et al., 2004; Griffin et al., 2007], while the effectiveness of reducing erosive tooth wear by fluoride is discussed controversially in the literature [Larsen and Richards, 2002; Wiegand and Attin, 2003; Amaechi and Higham, 2005].

During the last decade [Reynolds, 1997], the use of casein phosphopeptide/amorphous calcium phosphate (CPP-ACP) to enhance the remineralization of carious lesions and reduce the erosive potential of acidic drinks has been reported [Shen et al., 2001; Ramalingam et al., 2005]. Furthermore, a current study by Ranjitkar et al. [2009] showed statistically significant protective effects of CPP-ACP-containing crème against erosive/abrasive tooth wear. This effect, however, was not shown in other studies dealing with CPP-ACP-containing products [Lennon et al., 2006; Willershausen et al., 2009]. However, in these studies with controversial findings, erosion was elicited using solutions with pH values below the pH measured in the oral cavity during reflux events. During reflux events, intra-oral pH remains above 5.5 most of the time [Bartlett et al., 1996a]. Thus, information is lacking as to whether the use of CPP-ACP is able to minimize erosively induced hard tissue loss in patients suffering from gastro-oesophageal reflux disease.

The aim of the present study was to evaluate the degree to which a CPP-ACP-containing crème (GC Tooth Mousse) and different fluoride compounds (amine and sodium fluoride) can be used to prevent erosive/abrasive tooth wear in a gastro-oesophageal-reflux-simulating de-/remineralization and abrasion cycling process. The hypothesis of the present study, taking into consideration the findings of Ranjitkar et al. [2009], was that a CPP-ACP-containing crème can reduce tooth wear due to erosion/abrasion, to levels comparable with those seen when using amine and sodium fluoride compounds.

Materials and Method

Sample Preparation and Allocation

In the study, 40 enamel samples were prepared from 40 freshly extracted bovine lower incisors. The teeth were sectioned at the enamel/cementum junction with a water-cooled diamonded disc directly after the extraction. The pulp tissue was removed from the coronal part of the tooth with endodontic files. The crowns were placed in a cylindrical metal mould and embedded with acrylic resin (Palavit G, Kulzer, Wehrheim, Germany). After setting of the resin, the samples were removed from the moulds and the buccal surface of the crowns was ground with water-cooled carborundum discs (800, 1,000, 1,200, 2,400 and 4,000 grit; Water-Proof Silicon Carbide Paper, Struers, Erkrath, Germany) with water as coolant in an automatic grinding machine. During grinding, the outermost 200 μm of enamel was removed. This enamel loss was controlled with a micrometre (Mitutoyo, Tokyo, Japan). Two parallel indents were placed on the polished enamel using a scalpel blade. The space between the indents measured 1.5 mm. The enamel adjacent to the marked area served as a reference area for the surface profilometry. The Knoop microhardness of each sample was determined on the enamel surface of the reference areas. For this determination, 3 microhardness indentations were performed on the enamel surface, and the average of the 3 indentations was calculated. The samples were then stratified and allocated to 4 groups (1–4) according to their Knoop microhardness.

Experimental Procedure

From each sample, 5 baseline surface profiles were recorded (Perthometer S2, Mahr, Göttingen, Germany) with a distance of 100 μm between each profile. For exact repositioning of the samples after the later experimental procedure, the profilometer is equipped with a custom-made jig [Attin et al., 2009]. After the recording of the baseline profiles, the reference area was covered with tape (Tesa, Beiersdorf, Hamburg, Germany) to avoid any alterations of the enamel during the following de-/remineralization cycling or the toothbrush abrasion.

The de-/remineralization cycling was performed in an artificial mouth, previously presented in detail [Wiegand et al., 2009]. The whole de-/remineralization cycling ran for 20 days. Per day (8 h), 6 erosive attacks with HCl (pH 3.0) for 20 s were performed. Between the erosive attacks, the samples were perfused with artificial saliva. The artificial saliva was prepared following the formulation given by Klimek et al. [1982] and renewed each day. The flow rate of artificial saliva was 0.5 ml/min and that of HCl was 2 ml/min. Overnight the samples were stored in artificial saliva. Each day before the first and 1 h after the last erosive attack, the samples were brushed for 15 s with a toothpaste slurry in an automatic brushing machine applying reciprocating linear motion to the toothbrushes (ParoM43, Esro AG, Thalwil, Switzerland). The brushing machine was adjusted to a constant brushing frequency of 60 strokes/min and a constant brushing load of 2.5 N. The toothpaste slurry was prepared by mixing 300 ml artificial saliva and 100 ml toothpaste (Elmex, Gaba, Münchenstein, Switzerland). After the second toothbrushing of each day, the samples in group 2 were treated with an amine/sodium fluoride gel (Elmex Gelée, Gaba), while the samples in group 3 were treated with a neutral sodium fluoride gel (Paro Fluor Gelée, Esro AG, Kilchberg, Switzerland) and the samples in group 4 were treated with a CPP-ACP-containing crème (GC Tooth Mousse, GC Europe, Leuven, Belgium), later on referred to as CPP-ACP. Both fluoride gels had a concentration of 12,500 ppm. The pH of the amine/sodium fluoride gel measured 4.8 and that of the pure sodium fluoride gel 7.1. Samples in group 1 remained untreated and served as negative control. From each gel or crème, 2 ml were applied to each sample for 2 min. After the 20-day cycling, the tape was removed from the reference area of the samples and 5 new surface profiles/sample were recorded. The enamel wear was calculated by a custom-made software (4D Client, custom-designed software, University of Zürich, Zürich, Switzerland), allowing exact superimposition of the untreated, tape-protected, reference areas and comparing the baseline profiles with the respective posttreatment profiles. In a previous study, a lower measurement limit of 0.105 μm and a difference between 10 profiles at the same sample area of 0 ± 0.031 μm was determined for the stylus profilometer used here [Attin et al., 2009].

Statistical Analysis

For data presentation, the mean enamel wear of the 5 profiles/sample and the mean loss in each group were calculated. Statistical analysis was performed by ANOVA followed by the Bonferroni/Dunn post-hoc test for comparison of the experimental groups. The level of significance was set at 0.05, and the p values were adjusted by Bonferroni-Holm correction. Statistical analyses were done with Stat View (version 5.0.1, SAS Institute Inc., Cary, N.C., USA).

Results

Wear of enamel due to erosion/abrasion is given in figure 1. The highest enamel wear (0.352 ± 0.07 μm) due to erosion/abrasion was observed for group 1 (untreated/negative control). This enamel loss showed no statistically significant difference to the wear seen in the CPP-ACP group (group 4; 0.337 ± 0.178 μm; p = 0.7378). Enamel wear in group 2 (amine/sodium fluoride gel) was significantly lower than in group 1 (untreated/negative control) and group 4 (CPP-ACP group; p = 0.0007 and p = 0.0018, respectively), while enamel loss on the amine/sodium fluoride gel samples (group 2; 0.186 ± 0.047 μm) and the sodium fluoride gel samples (group 3; 0.182 ± 0.044 μm) showed no statistically significant difference to each other (p = 0.9333). A significantly lower amount of enamel wear due to erosion/abrasion was observed for group 3 (sodium fluoride gel group) compared to the control group 1 (untreated) and to group 4 (CPP-ACP group; p = 0.0006 and p = 0.0015, respectively).

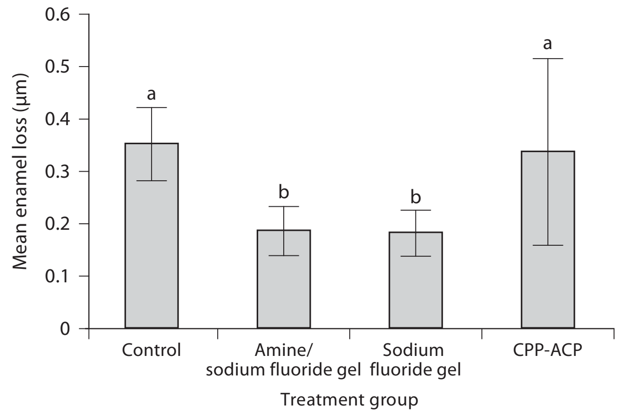

Fig. 1. Mean enamel loss and standard deviation due to erosion/abrasion in the 4 different treatment groups. Values that had no statistically significant difference are marked with the same superscript letters.

Discussion

In the present study, bovine enamel was used as a substitute for human enamel. Numerous other studies have used bovine enamel for erosion and erosion/abrasion tests for a variety of reasons [Meurman and Frank, 1991; Attin et al., 2001; Lennon et al., 2006; Rios et al., 2006; Vieira et al., 2006]. The main reason for using bovine enamel is that it is easier to obtain a sufficient number of sound bovine teeth [Oesterle et al., 1998]. Furthermore, the larger surface area of bovine lower incisors allows preparation of more than 1 sample/tooth, resulting in a reduction of intertooth differences. Previous studies have found no significant differences in either mineral content, mineral distribution or mechanical properties between human and bovine enamel [Davidson et al., 1973; Gente et al., 1985; Esser et al., 1998]. Other reasons for the use of bovine teeth are: bovine teeth often stem from cattle/cows from the same region, with similar environmental and nutrition factors. In contrast to human teeth, bovine teeth do not suffer from caries and do not have a history of fluoridation measures that might influence erosive tooth wear in an unknown manner. As 4 or even 6 bovine teeth can normally be harvested from 1 cow, bovine tooth samples are more homogenous than those gained from different human subjects.

The surface wear of enamel has been measured in the present study by the use of surface profilometry. This method has been used in numerous other studies concerning dental hard tissue wear due to erosion [West et al., 2000], abrasion [Wegehaupt et al., 2010] or erosion/abrasion [Attin et al., 2000a, b; Hooper et al., 2007]. Tooth wear has also been measured with other methods, such as assessment of the calcium diluted in the acid of the erosive attack [Wegehaupt et al., 2009b], calculating the wear due to the length change of previously applied Knoop indentations [Joiner et al., 2005; Wiegand et al., 2007] or measuring the amount of radioactive phosphate in the slurry after brushing radioactivated tooth structure [Imfeld, 2001]. These methods are used for the determination of tooth wear caused either by erosion or abrasion. As the enamel wear of the present study was caused by a combination of erosion and abrasion, wear could not be measured by the assessment of the calcium diluted in the acid, as the enamel wear was also caused by abrasion, which could not be determined with this method. The determination of the wear by the length change of Knoop indentation was not applicable either, as the application of acid would make the reading of the indentation length difficult and as it removes substance from the body of the indentation and therefore changes its appearance [Attin, 2006]. For these reasons and the fact that the method is well established in our laboratory, surface profilometry has been used, as it is capable of measuring wear due to both erosion and abrasion.

For this in vitro study, a demineralization/remineralization cycling with 2 toothbrushing episodes/day was performed. In contrast to other studies [Ganss et al., 2001; Lennon et al., 2006], HCl was used for demineralization in the present study. A HCl perfusion, 6 times/day for 20 s, was used to simulate the oral situation during regurgitation of stomach content when gastric juice reaches the oral cavity [Hunt, 1951]. After contact with the HCl, the samples were immediately perfused with artificial saliva. As the flow rate of the artificial saliva was much lower than that of the acid, one might assume that the pH drop below 5.5 might have lasted longer than 20 s/erosive attack, thus resulting in a total time of pH under 5.5 of over 120 s/day in the present study. This assumption was verified during preliminary tests, by measuring the pH in the collected mixture of artificial saliva and HCl after leaving the chamber of the artificial mouth. Bartlett et al. [1996a] found a drop of the oral pH below 5.5 during 0.3% and below pH 6 during 4.4% of the total time during 24-hour pH telemetry in gastro-oesophageal reflux patients, which means an erosion time between 4.3 and 60 min/day. As enamel erosion occurs at a pH of approximately 5.5, depending on the concentration of calcium and phosphate in the saliva [Gudmundsson et al., 1995], the respective data of Bartlett et al. [1996a] was used as an orientation to estimate the total duration of the erosive attack per day in the present study. A limitation of the present cycling model might be that no gastro-oesophageal reflux was simulated overnight, as reflux is not only limited to the daytime but also occurs during the night [Bartlett et al., 1996b]. Beside HCl, the gastric juice also contains various enzymes [Hunt, 1951] such as pepsin. In the present study, the acid was not admixed with the proteolytic enzyme pepsin, as it had been in a previous study [Schlueter et al., 2007]. The amount of organic matrix in enamel is very low (0.035%) [Belcourt and Gillmeth, 1979] and even for dentine, no influence of pepsin admixture on the erosive/abrasive tooth wear could be observed. Toothbrushing was performed before erosion and 1 h after the last erosive attack per day to follow the recommendations made for patients suffering from erosion [Lazarchik and Filler, 1997; Wiegand et al., 2008], although there are studies showing that the period between erosion and toothbrushing has only a minor effect on the erosion/abrasion tooth wear [Ganss et al., 2007].

The hypothesis of the present study had to be rejected. In contrast to the findings of Ranjitkar et al. [2009], no protective effect of CPP-ACP-containing mousse on erosive/abrasive tooth wear was observed in the present study. One reason for these contradictory findings might be that a non-fluoridated toothpaste was used in the earlier study, resulting in an overestimation of the effect of CPP-ACP-containing mousse in reducing erosive tooth wear, as suggested by the authors of that article. Furthermore, in that study, the eroded samples were brushed immediately after the erosive attack without any time for remineralization to occur. The CPP-ACP mousse was applied before each erosion/brushing cycle, thus resulting in a much higher frequency of application, compared to the present study.

The data of the present study showed a significant reduction of erosive/abrasive tooth wear due to the application of amine/sodium and pure sodium fluoride gels. This finding is in agreement with the findings of other studies [Attin et al., 1999; Lagerweij et al., 2006]. Taking into consideration that previously eroded enamel is more susceptible to abrasion than sound enamel [Attin et al., 2000a, b], one might assume that the prevention of erosion will also result in a reduction of erosive/abrasion tooth wear. Although there are studies showing no positive effect of neutral sodium fluorides on the protection of erosion, there are other studies showing a protective effect of sodium fluoride [van Rijkom et al., 2003]. These differences might be attributed to different erosion protocols or different preparations of the fluoride compounds. Furthermore it has to be taken into consideration that in the present study the enamel samples were eroded before the fluoride gels were applied. This erosion, prior to fluoride application, causes an enlargement of the enamel surface able to react with the fluoride compounds [Attin et al., 2000a, b], thus making the pH of the applied fluorides less important.

There is a broad consensus that fluoride protects enamel against erosive tooth wear [Wegehaupt et al., 2009a], but the mechanisms by which CPP-ACP reduces erosive tooth wear are still unclear [Ranjitkar et al., 2009]. Tantbirojn et al. [2008] suggest that the erosion-inhibiting potential of CPP-ACP probably involves remineralization by deposition of mineral into the porous zone of the eroded enamel [Ranjitkar et al., 2009]. This might lead to the hypothesis that the protective effect of CPP-ACP-containing products is only valid if the erosion has already occurred. Despite this being the case in the present study, no protective effect of the CPP-ACP-containing mousse was observed. Therefore it might be postulated that the application frequency of the CPP-ACP-containing mousse must be greater than that used in the present study. This suggestion is also supported by the findings of Ranjitkar et al. [2009].

Within the limitations of this in vitro study, it might be concluded that erosive/abrasive tooth wear, caused by frequent HCl exposure and toothbrushing, could be reduced significantly by the daily application of fluoride gels, irrespective of their fluoride compound, while the single application per day of CPP-ACP-containing mousse is less effective. Further studies are needed to confirm whether increasing the number of applications per day could enhance the preventive effect of the CPP-ACP-containing mousse.

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